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<p align="center">Division of Forensic Science</p> <p align="center">CONTROLLED SUBSTANCES TRAINING MANUAL</p>	<p>Amendment Designator: A</p>
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<p align="center">8 MARIJUANA AND ITS ANALYSIS</p> <p>8.1 Introduction</p> <p>Because the analysis of marijuana is relatively simple and somewhat different from the analysis of other drugs, it is advantageous to teach the trainee this procedure first. It not only gives the new chemist a feeling of accomplishment but also provides the coordinator with an opportunity to view the trainee's analytical techniques.</p> <p>8.2 Objectives</p> <p>8.2.1 To familiarize the trainee with the protocol for marijuana</p> <p>8.2.2 To make the trainee proficient in the analysis and identification of marijuana and marijuana products</p> <p>8.3 Modes of Instruction</p> <p>8.3.1 Self-directed study through reading assignments and worksheets</p> <p>8.3.2 Presentations and demonstrations</p> <p>8.3.3 Practical exercise</p> <p>8.4 Reference</p> <p>8.4.1 Moffat, A.C. editor <i>Clarke's Isolation and Identification of Drugs</i>. London: The Pharmaceutical Press, 1986, pp. 423-425.</p> <p>8.4.2 Nakamura, George R. and Thorton, John I. "The Identification of Marijuana" <i>Journal of the Forensic Science Society</i>, (1972), 12, 461.</p> <p>8.4.3 Virginia Division of Forensic Science Drug Analysis Procedure Manual, Marijuana Section</p> <p>8.4.4 AOAC Methods (1980) Section 40.012 and 40.013 (page 686)</p> <p>8.4.5 <i>Drugs of Abuse</i>, DEA Publication, 1997.</p> <p>8.4.6 Bailey, K. "The Value of the Duquenois Test of Cannabis – A Survey" <i>Journal of Forensic Science</i>, (1971), 24(4), pp. 817-841.</p> <p>8.4.7 Nakamura, G. R. "Forensic Aspects of Cystolith Hairs of Cannabis and Other Plants." <i>Journal of the AOAC</i>, (1969), Vol. 52, No. 1, pp. 5-16.</p> <p>8.4.8 Bureau of Narcotics, <i>Marihuana, Its Identification</i>, United States Government Printing Office, Washington, 1948.</p> <p>8.4.9 Nakamura, George R. and Thorton, John I. "The Forensic Identification of Marijuana: Some Questions and Answers". <i>DEA</i> (1973), Vol. 1, pp. 102-112.</p> <p>8.4.10 Smith, R.N. "Brief Note on the Response of Some Essential Oils and Extracts of Vegetable Origin to the Duquenois-Levine Test for Cannabis." <i>Journal of the Forensic Science Society</i>, (1974), 14, pp. 191.</p> <p>8.4.11 Waller, C.W. The Chemistry of Marijuana. <i>Proc. West. Pharmacol. Soc.</i>, (1971), 14, pp. 1-3.</p> <p>8.4.12 Segelman, A. B. "The Rim Test: A Reliable and Useful Procedure for the Detection and Identification of Marijuana Utilizing Combined Microscopy and Thin-Layer Chromatography" <i>Journal of Chromatography</i>,</p>	

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<p>(1973), 82, pp. 151-157.</p> <p>8.4.13 Grinspoon, L. "Marijuana" <i>Scientific American</i>, (1969), Vol. 221, No. 6, p. 17.</p> <p>8.4.14 Pitt, C. G., et al. "The Specificity of the Duquenois Color Test for Marihuana and Hashish" <i>Journal of Forensic Science</i>, 1972, p. 693.</p> <p>8.5 Assignments</p> <p>8.5.1 Completion of required reading assignments (8.4.2, 8.4.3, 8.4.7, 8.4.14)</p> <p>8.5.2 Practical exercises</p> <p>8.5.3 Study questions</p> <p>8.6 Study Questions</p> <p>8.6.1 What is the definition of marijuana as per the Code of Virginia? How is it scheduled?</p> <p>8.6.2 Compare and contrast the definition from the Code of Virginia to that in the Federal Controlled Substances Act. What are the possible analytical implications?</p> <p>8.6.3 Describe the appearance of a mature marijuana plant.</p> <p>8.6.4 What is the derivation of the word "marijuana"?</p> <p>8.6.5 What are Charas, Ganga, Bhang and Kif?</p> <p>8.6.6 What is sinsemilla and how is it grown?</p> <p>8.6.7 What is hemp?</p> <p>8.6.8 What is the scientific name for marijuana including family, genus and species?</p> <p>8.6.9 What parts of the plant contain THC?</p> <p>8.6.10 Define "agronomic variety" and differentiate between Cannabis sativa, Cannabis ruderalis, Cannabis indica and Cannabis americana.</p> <p>8.6.11 Define "dioecious" and relate to Cannabis. Include the different morphological characteristics between the two.</p> <p>8.6.12 What is the function of the resin found on the plant?</p> <p>8.6.13 Describe the differences between hashish and hash oil including preparation, schedule, and analysis.</p> <p>8.6.14 What is the average percentage of THC found in marijuana?</p> <p>8.6.15 What is the pharmacological classification of marijuana?</p> <p>8.6.16 Define the following:</p> <ul style="list-style-type: none"> • cannabinoid • alkaloid • cis and trans isomers 	

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<div style="margin-left: 40px;"> <ul style="list-style-type: none"> • optical isomers • parahexyl • synhexyl • dronabinol </div> <p>8.6.17 What factors influence the relative amounts of cannabinoids present in marijuana?</p> <p>8.6.18 What are the two numbering systems for cannabinoids in use today. Draw THC and show how these numbering systems differ.</p> <p>8.6.19 What types of isomers are Δ^9-THC and Δ^8-THC? Which is the more stable?</p> <p>8.6.20 Is d- or l-THC the naturally occurring isomer?</p> <p>8.6.21 Are the cannabinoids acidic or basic? Polar or non-polar?</p> <p>8.6.22 Chemically, can any of the other cannabinoids break down or be converted to THC? Does THC break down?</p> <p>8.6.23 What information is gained from the macroscopic examination?</p> <p>8.6.24 What information can be obtained from the microscopic examination?</p> <p>8.6.25 What power of magnification is needed to view cystolithic hairs and glandular hairs?</p> <p>8.6.26 Describe cystolithic hairs including characteristics and locations found on marijuana.</p> <p>8.6.27 Describe the HCl test including when to perform it and what information is gathered.</p> <p>8.6.28 Describe glandular hairs including characteristics and locations found on marijuana.</p> <p>8.6.29 How can you distinguish between marijuana cystoliths and cystoliths on other plants?</p> <p>8.6.30 Discuss any other plants which have cystoliths including how to differentiate them from those found on marijuana such as:</p> <div style="margin-left: 40px;"> <ul style="list-style-type: none"> • <i>Humulus japonica</i> • <i>Humulus lupulus</i> • <i>Lantana camara</i> </div> <p>8.6.31 What is a screening test?</p> <p>8.6.32 What is the RIM test?</p> <p>8.6.33 Describe the Duquenois-Levine (D-L) procedure as published by the AOAC and discuss any differences from the procedure used in the Division. Include any references which validate the DFS procedure.</p> <p>8.6.34 What substances give false positive results for each of the three tests? Be sure to address the following substances:</p> <div style="margin-left: 40px;"> <ul style="list-style-type: none"> • <i>Ribes viburnifolium</i> (currant) • <i>Myristica fragrans</i> (mace) • Olivetol • Coffee • Patchouli oil </div>	

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<div data-bbox="316 296 534 422"> <ul style="list-style-type: none"> • Pennyroyal oil • Peppermint oil • Sandalwood oil • Parahehyl </div> <div data-bbox="217 457 1507 512"> 8.6.35 What causes the purple color obtained with the Duqueois reagent and marijuana? What determines whether this product is soluble in the chloroform? Describe/draw a probable reaction mechanism. </div> <div data-bbox="217 548 1453 575"> 8.6.36 Describe how Pitt used a spectrophotometer to evaluate colors produced by the D-L test. Is this necessary? </div> <div data-bbox="217 611 1044 638"> 8.6.37 Describe the following tests including their uses and positive results: </div> <div data-bbox="316 674 698 926"> <ul style="list-style-type: none"> • meta-Duquenois test • Beam test • Ghamrawy reaction • Duquenois test • Duquenois-Negm test • Modified Duquenois test • D-L test • Rapid modified Duquenois test </div> <div data-bbox="217 961 1240 989"> 8.6.38 Define thin layer chromatography (TLC) including how the test is normally performed. </div> <div data-bbox="217 1024 1461 1079"> 8.6.39 Describe the chromatography plates used in the lab including the purpose of each component. Why is silica generally preferred over alumina? </div> <div data-bbox="217 1115 980 1142"> 8.6.40 Define the following chromatography terms as related to TLC: </div> <div data-bbox="316 1178 537 1367"> <ul style="list-style-type: none"> • R_f • solvent front • elutropic series • theoretical plate • resolution • chromophore </div> <div data-bbox="217 1402 1539 1430"> 8.6.41 What are Fast Blue B and Fast Blue BB including their chemical names and the advantages/disadvantages of each? </div> <div data-bbox="217 1465 1008 1493"> 8.6.42 Explain the chemical basis of TLC covering the following topics: </div> <div data-bbox="316 1528 1494 1682"> <ul style="list-style-type: none"> • Types of chromatography • Different stationary phases • Interactions among the stationary phase, mobile phase and solute, including consideration of equilibrium • Influences on chromatography/separation by stationary phase thickness, temperature, humidity, molecular weight, gravity, polarity of mobile phase </div> <div data-bbox="217 1717 1370 1745"> 8.6.43 What standards are used with marijuana TLC analysis and to what level have they been confirmed? </div> <div data-bbox="217 1780 1453 1835"> 8.6.44 What mobile phase(s) are preferred for marijuana analysis? Include any references which validate the DFS procedure. </div> <div data-bbox="217 1871 1453 1925"> 8.6.45 Describe the TLC visualization results obtained by either Fast Blue B or Fast Blue BB with the three major cannabinoids. </div>	

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<p>8.6.46 Describe the specificity of the three tests normally incorporated into the analytical scheme for marijuana analysis (i.e. Microscopic, Thin-Layer Chromatography and D-L). What other tests are available for the analysis of marijuana and when are they necessary?</p> <p>8.6.47 Describe the quality assurance procedures for the D-L reagents and the TLC baths and sprays.</p> <p>8.6.48 What are the detection limits of the three main tests?</p> <p>8.6.49 Define “residue” as it relates to marijuana analysis. Describe an appropriate procedure for analyzing a smoking device containing suspected marijuana residue.</p> <p>8.7 Practical Exercises</p> <p>8.7.1 Examine each of the following under the microscope and describe in detail:</p> <ul style="list-style-type: none"> • Dry marijuana leaf material • Sinsemilla • Marijuana seeds • Marijuana stems • Hashish • Hash oil • Hops • Oregano • Tobacco • Sage • Parsley • Ditchweed, if available • Burr Marigold, if available • Salvia Divinorum, if available <p>8.7.2 Perform Duquenois-Levine tests on the following and describe results:</p> <ul style="list-style-type: none"> • Marijuana • Hashish • Hash oil • Patchouli oil • Oregano • Parsley • Coffee • Hops • Tobacco • Olivetol • Δ9-THC • Cannabinol • Cannabidiol • Resorcinol • Dragon’s Blood incense, if available <p>8.7.3 Perform TLC analysis of the following using the marijuana bath and the other baths used in the laboratory:</p> <ul style="list-style-type: none"> • Marijuana • Δ9-THC 	

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<div data-bbox="315 296 756 714"> <ul style="list-style-type: none"> • Δ8-THC • Cannabinol • Cannabidiol • Patchouli oil • Hash oil • Hops • Tobacco • Oregano • Parsley • Coffee • Olivetol • Resorcinol • Dragon's Blood incense, if available </div> <div data-bbox="217 745 1403 777"> <p>8.7.4 Perform GC/MS analysis of the following, noting retention times and major mass spectral differences:</p> </div> <div data-bbox="315 810 617 999"> <ul style="list-style-type: none"> • Marijuana leaf material • Δ9-THC • Δ8-THC • Cannabinol • Cannabidiol • Olivetol </div> <div data-bbox="217 1033 1403 1064"> <p>8.7.5 Prepare hash oil and quantitate the Δ9-THC using the method outlined in the DFS Procedures Manual.</p> </div> <div data-bbox="217 1096 1542 1155"> <p>8.7.6 Obtain marijuana seeds from the Training Coordinator. Germinate the seeds and analyze the young plant using the normal analytical scheme. Note any difficulties or differences between young plants and more mature plants.</p> </div> <div data-bbox="217 1186 842 1218"> <p>8.7.7 Analyze Dronabinol including GC/MS and FTIR.</p> </div> <div data-bbox="217 1249 1528 1306"> <p>8.7.8 Obtain a sample of charred marijuana residue from the TC. Analyze using the analytical scheme listed in the DFS Procedures Manual.</p> </div> <div data-bbox="217 1339 1533 1396"> <p>8.7.9 Receive mock cases from the TC. Work these as real cases including the preparation of a Certificate of analysis to be used in mini-mock trials.</p> </div> <div data-bbox="217 1430 786 1461"> <p>8.7.10 Participate in at least three mini-mock trials.</p> </div> <div data-bbox="149 1491 454 1522"> <p>8.8 Modes of Evaluation</p> </div> <div data-bbox="217 1551 535 1583"> <p>8.8.1 Written examination</p> </div> <div data-bbox="217 1614 470 1646"> <p>8.8.2 Court exercise</p> </div> <div data-bbox="1474 1644 1542 1675"> <p align="right">♦ End</p> </div>	